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ScienceDirect

Energy Procedia 136 (2017) 369–373

Energy Procedia

www.elsevier.com/locate/procedia

4th International Conference on Energy and Environment Research, ICEER 2017, 17-20 July 2017, Porto, Portugal

Analyzing *Phaeodactylum tricornutum* lipid profile for biodiesel production

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Abstract

Microalgae are eukaryotic or prokaryotic organisms, with high photosynthetic efficiency for biomass production. The constituent elements of microalgae can be extracted and used as raw material for the production of various products, such as biodiesel, which the main components are fatty acid methyl esters (FAMEs). This study aimed to analyze the lipids composition of *Phaeodactylum tricornutum* cultured at outdoor photobiorreactor. The *P. tricornutum* samples showed 24.39% of C16-C18 fatty acids, 26.52% saturated fatty acids (SFAs), 21.91% monounsaturated fatty acids (MUFAs) and 32.02% polyunsaturated fatty acid (PUFA). These findings suggest that *P. tricornutum* lipids meet to international biodiesel standards and could be an alternative raw material for biodiesel production.

Peer-review under responsibility of the scientific committee of the 4th International Conference on Energy and Environment Research.

Keywords: Biodiesel; lipid extraction; microalgae cultivation

1. Introduction

Microalgae are sunlight-driven cell factories that convert carbon dioxide into potential biofuels, foods, feed, and high-value bio-actives. Compared with traditional crops, they have a high areal productivity, a relatively high oil

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1876-6102
Peer-review under responsibility of the scientific committee of the 4th International Conference on Energy and Environment Research.
10.1016/j.egypro.2017.10.251

and protein content, and do not depend on arable land and freshwater. Nowadays, there is a strong interest in lipid production using microalgae. Microalgae are theoretically capable of producing much more lipids than any conventional crops and are, therefore, attractive as a potential feedstock for biodiesel [1].

Among microalgae, diatoms are responsible for about 40% of the marine primary productivity [2] and despite their abundance, diversity and easiness to cultivate, few of them are cultured for biotechnology purposes [3]. Moreover, the marine microalgae are especially attractive to cultivate for producing biotechnological products due to the fact that they do not compete with fresh water sources.

The marine diatom *Phaeodactylum tricornutum* has been selected as a potential strain for biofuels production [4,5] and other added value products, as carotenoids and eicosapentanoic acid (EPA), both of them largely used for aquaculture animal feed and for human consumption, as well. Moreover, *Phaeodactylum* grows in saline water, and does not compete with fresh water sources, which makes it commercially more competitive [6,7].

FAMEs are the main constituent of biodiesel. Although, FAMEs have been studied recently by some authors, there is still incipient data reported about the fatty acid profiles of the microalgae oils [8,9].

The aim of this work was the analysis of the culture growth and fatty acids composition of *P. tricornutum* cultivated at an outdoor prototype photobioreactor for biodiesel production under natural Chilean conditions.

2. Material and methods

2.1. Microalgae cultivation

The inoculum of marine microalgae *Phaeodactylum tricornutum* was batch cultured in natural seawater in four 20 L polycarbonate flasks (carboys) under artificial light with 200 µmol photons m^{-2} s^{-1} in a 16:8 (light:dark) cycle at 23 ± 1 °C. The Walne medium was used, and had the following components (in mg/L): $FeCl_3.6H_20$ 1.3, $MnCl_2.2H_20$ 0.36, H_3BO_3 33.6, EDTA 45.0, $NaH_2PO_4.2H_20$ 20.0, $NaNO_3$ 100.0, $Na_2SiO_3.9H_2O$ 30.0, supplied with 100 µL of vitamin solution (Vitamin B12 100.0 mg/L Vitamin B1 100.0 mg/L, Vitamin H 2000 µg/L). Mixing and aeration was facilitated by use of an aquarium pump. After 14 days of cultivation, the inoculum was transferred to four outdoor 200 L bubble column photobioreactors. Algal cells were harvested by flocculation with 0.5 M NaOH, pH 10.40 overnight, followed by biomass concentrate centrifugation at 4000 rpm for 5 minutes. The biomass was lyophilized at -70 ± 2 °C for about 72 hours and stored at -20 °C.

2.2. Lipid extraction and CG analysis

Lipids were extracted from samples by using Bligh & Dyer method [10]. Lipid composition was determined based on the FAME profile. The FAME mixture was prepared directly from the dried algal biomass. Briefly, the transesterification process was performed using 2 mL of BF3 in methanol (2 wt.%) at 100 ºC for 30 minutes to determine FAME content. After reaction completion and cooling the reaction mixture, 1 mL of isooctane was added, followed by shaking. 5 mL of saturated NaCl were added and centrifugation was performed. The upper phase was carefully transferred to 2 mL amber vials, and stored at -20 ºC. The chromatographic analysis was performed using a gas chromatograph–mass spectrometer (Carlo Erba Instruments, GC 6000 Vega Series 2, model 6300-03b).

3. Results and discussion

3.1. Biomass cultivation

The *P. tricornutum* outdoor batch culture were monitored daily by measurements of biomass concentration, temperature, pH and salinity. Fig. 1 shows that the highest biomass concentration (0.96 kg·m^3) was obtained in stationary phase at day 12 of cultivation. The temperature varied from 17 to 21 °C, pH ranged from 7 to 9 and salinity remained almost constant during all cultivation period. Interesting, on day 9, when the temperature starts decreasing the biomass concentration does not vary anymore. At this point the culture was at the stationary phase and was harvested.

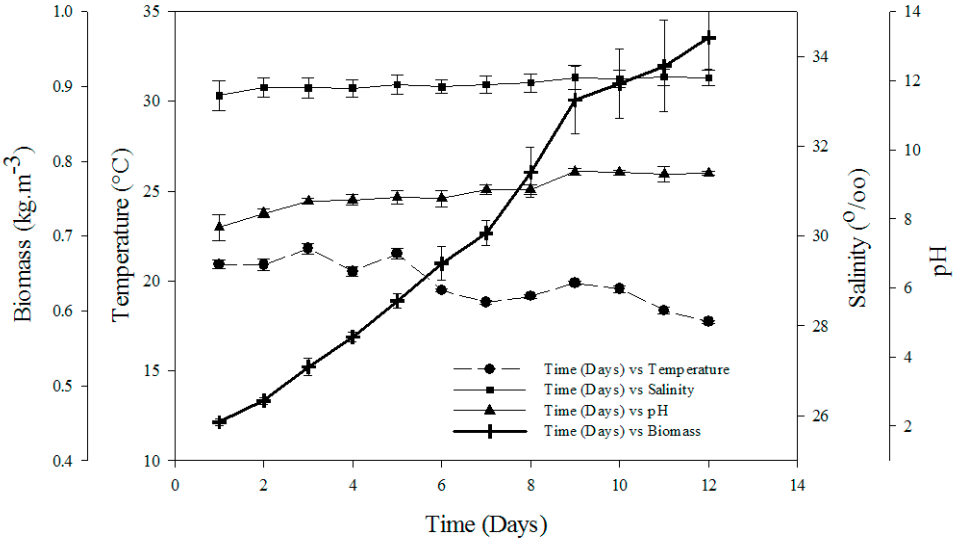

Fig. 1. Variation of culture parameters for *P. tricornutum* batch culture.

3.2. *FAMEs analysis*

The composition and relative abundance of each microalgae fatty acid was estimated based on CG area signals (% area per sample) for the corresponding FAME, it is shown in Fig 2. FAME mainly contains saturated and unsaturated carbon chain lengths from C11 to C24. The biggest component fraction is the Eicosatrienoic acid (C20:3), with relative abundance of 29.69%, followed by 12.06% of the Pentadecanoic acid (C15:0), 13.43% of the unsaturated Pentadecenoic acid (C15:1), 15.82% of the Heptadecanoic acid (C17:0), 8.94% of the Decanoic acid (C12:0), 4.89% of Palmitoleic acid (C16:1), 3.18% of Stearic acid (18:0), 2.47% of Oleic acid (18:1), 1.12% of Heptadecenoic acid (C17:1). It was found low levels of Linoleic acid (C18:2) with 0.67%, followed by 0.61% of Docosadienoic acid (C22:2), 0.54% of Arachidonic acid (C24:4), 0.51% of Eicosadienoic acid (C20:2) and 0.07% of Undecanoic acid (C11:0) (shown in Fig 2).

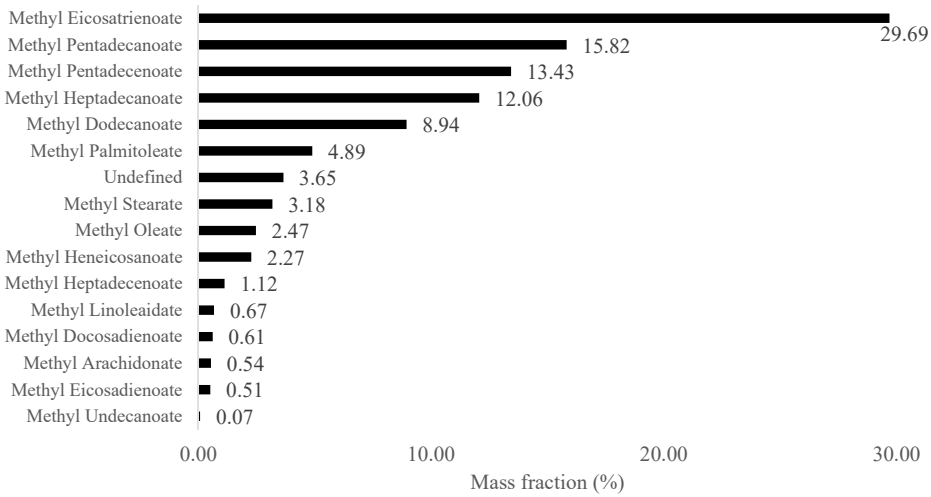

Fig. 2. Fatty acid weight profiles of *P. tricornutum* (% of total fatty acid).

Analysis of FAMEs profile plays a most important role in determining fuel properties. Furthermore, the occurrence of C16-C18 fatty acids is considered as good composition for biodiesel production [11]. The *P. tricornutum* samples showed 24.39% of C16-C18 fatty acids, which can provide the finest relation between oxidative stability and cold flow properties [11]. Concerning the presence of SFAs and MUFAs, the samples showed a proportion of 26.52% and 21.91%, respectively. Other studies [12] have shown a higher proportion of these class of fatty acids (SFAs - 50.16% and MUFAs - 48.79%) but this enhancement was mainly due to using different gas liquid ratios, although the relation between SFAs and MUFAs were similar in our findings. These findings suggest that *P. tricornutum* oils could achieve high cetane number and low iodine value, which complies with the European (EN 14214) and US (ASTM D6751) standards requirements [13,14].

The FAMEs profile showed that *P. tricornutum* contained considerable amounts of PUFAs (32.02%). The *P. tricornutum* PUFA`s production have been reported for outdoor cultures, and the findings suggested under nutrient replete conditions the amount of PUFAs reached about 60% [15]. Although, our studies were conducted outdoor under nutrient replete conditions, the amounts of PUFAs were lower, which might be due to different temperature and lighting conditions. The European standard for biodiesel (EN 14214) requires less than 1% of highly polyunsaturated fatty acids (≥4 double bonds), which could influence fuel properties of the resulting biodiesel. Although the high concentration of PUFA at the *P. tricornutum* FAMEs showed low values (0.54%) for Arachidonic acid (C24:4), a highly polyunsaturated fatty acid present in this microalgae oil.

4. Conclusions

The biomass of *P. tricornutum* was cultured in outdoor photobioreactors under Chilean conditions during 12 days and the final dry biomass concentration was 0.96 $kg.m^3$. The extracted lipids were transesterified, the FAMEs profile was analyzed and the composition of the fatty acids showed a proportion of 26.52% of SFAs, 21.91% of MUFAs and 32.02% of PUFAs. This profile meets the requirements of international biodiesel standards, showing that it could be a good alternative for biodiesel production. However, it is necessary further studies to assess FAME content of different microalgae species to determine the potential for biodiesel production, considering different climate and stressful conditions, since microalgae can change their fatty acids profile under diverse farming environments.

Acknowledgements

Authors acknowledge “Convenio de Desempeño Ciencia, Tecnología e Innovación para la Bioeconomía”, from Chilean government, for their support throughout this work. Authors also acknowledge the financial support from PFRH Petrobrás (Brazil) and European Commission (through Erasmus Mundus Program) post graduate research scholarship.

Authors thank the financial support of project POCI-01-0145-FEDER-006939 (Laboratory for Process Engineering, Environment, Biotechnology and Energy – LEPABE) funded by FEDER through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI) and by national funds through FCT.

References

[1] Halim R, Danquah MK, Webley PA. Extraction of oil from microalgae for biodiesel production: A review. Biotechnol Adv 2012;30:709–32.
[2] Silva Benavides AM, Torzillo G, Kopecký J, Masojídek J. Productivity and biochemical composition of Phaeodactylum tricornutum (Bacillariophyceae) cultures grown outdoors in tubular photobioreactors and open ponds. Biomass and Bioenergy 2013;54:115–22.
[3] Chauton MS, Olsen Y, Vadstein O. Biomass production from the microalga Phaeodactylum tricornutum: Nutrient stress and chemical composition in exponential fed-batch cultures. Biomass and Bioenergy 2013;58:87–94.
[4] Chisti Y. Biodiesel from microalgae beats bioethanol. Trends Biotechnol 2008;26:126–31.
[5] Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: A review. Renew Sustain Energy Rev 2010;14:217–32.
[6] Mata TM, Martins AA, Caetane NS. Advances in Biodiesel Production. Elsevier; 2012.
[7] Huang G, Chen F, Wei D, Zhang X, Chen G. Biodiesel production by microalgal biotechnology. Appl Energy 2010;87:38–46.
[8] Han F, Wang W, Li Y, Shen G, Wan M, Wang J. Changes of biomass, lipid content and fatty acids composition under a light–dark cyclic culture of Chlorella pyrenoidosa in response to different temperature. Bioresour Technol 2013;132:182–9.

[9] Wu C, Wang W, Yue L, Yang Z, Fu Q, Ye Q. Enhancement effect of ethanol on lipid and fatty acid accumulation and composition of Scenedesmus sp. Bioresour Technol 2013;140:120–5.
[10] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911–7.
[11] Knothe G, Steinbüchel A, Steinbüchel A, Roessler PG, Hidalgo JM, Luna D, et al. Improving biodiesel fuel properties by modifying fatty ester composition. Energy Environ Sci 2009;2:759.
[12] Song M, Pei H, Hu W, Han F, Ji Y, Ma G, et al. Growth and lipid accumulation properties of microalgal Phaeodactylum tricornutum under different gas liquid ratios. Bioresour Technol 2014;165:31–7.
[13] Hoekman SK, Broch A, Robbins C, Ceniceros E, Natarajan M. Review of biodiesel composition, properties, and specifications. Renew Sustain Energy Rev 2012;16:143–69.
[14] Refaat AA. Correlation between the chemical structure of biodiesel and its physical properties. Int J Environ Sci Technol 2009;6:677–94.
[15] Rodolfi L, Biondi N, Guccione A, Bassi N, D'Ottavio M, Arganaraz G, et al. Oil and eicosapentaenoic acid production by the diatom *Phaeodactylum tricornutum* cultivated outdoors in Green Wall Panel (GWP?) reactors. Biotechnol Bioeng 2017.